A case of apparent trisomy 21 without the Down's syndrome phenotype

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Abstract

We describe a case of apparent trisomy 21 that does not fulfil the criteria for the clinical diagnosis of Down's syndrome (DS). Our patient was subjected to karyotype analysis and found to have full, non-mosaic trisomy 21 in both blood lymphocytes and skin fibroblasts, while examination of the term placenta, which was performed earlier in the course of a different study, had shown mosaicism (73%) for trisomy 21. FISH analysis showed no obvious rearrangement of the DS chromosomal region in any of the chromosomes 21. Molecular analysis using polymorphic markers on chromosome 21 verified the existence of trisomy for the entire long arm of the chromosome and showed that the origin of the extra chromosome was maternal and was probably the result of a mitotic error. In contrast with the above, the clinical evaluation using the Jackson checklist of 25 signs failed to establish the diagnosis of DS. We believe that our patient might present mosaicism in other tissues that are not available for analysis and can be regarded as an extreme example in the continuous spectrum of karyotype/phenotype associations in mosaic cases.

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Down's syndrome (DS) has been described as a clinical entity since 1866 and is a well defined syndrome with a variety of phenotypic features present with different frequencies in affected subjects.[1] Some more consistent features are mental retardation, brachycephaly, ear abnormalities, flat nasal bridge, oblique eye fissures, protruding tongue, muscular hypotonia, etc. DS has a frequency of 1 in 650-1000 live births[2] and has been associated with trisomy for chromosome 21 since 1959.[3] So far most DS patients have been found to have full or partial trisomy for chromosome 21, while only in a few cases has no detectable duplication been identified.[4] In 2 to 4% of cases the trisomy for chromosome 21 occurs as mosaicism with a normal cell line.[2] Many attempts have been made to associate different phenotypic features with duplication of different parts of the chromosome by studying partial trisomies,[4–8] the results of which have indicated that most of the features can be correlated with trisomy of a small region around locus D21S55. This region has been referred to by many authors as the Down's syndrome chromosomal region (DCR), although genes outside the region might contribute to the full syndrome.[9]

A reliable checklist system has been described for the diagnosis of DS based on 25 phenotypic features.[10] In 100% of cases diagnosed using the checklist (13 or more signs were present) trisomy 21 was subsequently found cytogenetically.[10] In subjects with fewer than five signs, a normal karyotype was unambiguously predicted.[10] Although karyotype analyses have been done extensively during the last 30 years on subjects both with and without the specific DS phenotype, to the best of our knowledge no case of full trisomy 21 not presenting the DS phenotype has been reported so far. We describe here a case of a female infant who has a mild phenotype that does not fulfil the minimum Jackson criteria for the diagnosis of DS, although she was found to have non-mosaic trisomy 21 in lymphocytes and skin fibroblasts.

Materials and methods

CASE REPORT

The patient was examined twice independently. The first examination was during a study on the possible association of growth retardation in otherwise normal children with confined placental mosaicism.[11] The patient had mosaic trisomy 21 in the placenta but was finally not included in that study because of the slightly abnormal phenotypic features that she presented. The patient was referred to us at the age of 1 week because of her phenotypic features. At 2 months, 4 years, and 4.7 years of age, careful evaluation of the phenotype, karyotype analysis in blood lymphocytes and fibroblasts, and molecular analysis on DNA from blood were performed. The mental status of the patient has unfortunately been influenced by *Haemophilus influenzae* meningitis that occurred at the age of 12 months.

CYTOGENETIC ANALYSIS

Chromosome analyses were performed after standard preparation of fibroblast cultures and PHA stimulated lymphocyte cultures with subsequent GTG and QFQ banding, respectively. Long term placental cultures and chromosome preparations were established as described elsewhere.[12]

FISH ANALYSIS

Metaphase chromosomes were obtained from skin fibroblasts from the patient. Cosmid probes (1 μg of each) were labelled by nick

translation using biotin-14-dATP (BRL), purified by passage through G50 Sephadex, and precipitated with 3 mol/l sodium acetate in the presence of salmon sperm DNA and ethanol. Probes were dissolved in 40 µl hybridisation buffer with 100 fold human DNA as competitor and denatured at 100°C for 10 minutes. In situ hybridisation was performed according to a previously described protocol,[13] and antibiotin FITC conjugated antibody was used for detection. Chromosome preparations were counterstained with propidium iodide and examined with a Zeiss Axiophot microscope.

Cosmid c103E0669 was isolated by screening a chromosome 21 specific cosmid library with the probe D21S395.[14] Cosmids LLNL35A7 and LLNL51G9 have been described and localised in other reports[15] (Delabar *et al*, unpublished data).

MOLECULAR ANALYSIS

Genomic DNA from blood lymphocytes of the proband and her parents was extracted using standard procedures.[16] The DNA was used for polymerase chain reaction (PCR) amplification of polymorphic short sequence repeat sequences (microsatellites). The polymorphic alleles were visualised after end labelling of primer, electrophoresis of the radiolabelled PCR products through denaturing acrylamide gels, and autoradiography, as previously described.[17 18] The scoring of polymorphic alleles was performed as previously described[19] using numbers beginning with the shorter and increasing towards the longer alleles. Microsatellite primer sequences and PCR conditions have been published or referenced elsewhere.[20-23]

Results

Clinical photographs of the patient aged 15 days and 3.5 years are shown in fig 1. The clinical evaluation at the age of 4 years showed that the patient clearly had only two of the phenotypic features listed in the Jackson list of 25 signs of DS, that is, brachycephaly and flat nasal bridge (table 1). Epicanthic eye folds were discrete. The incisors were small, and the fifth finger was not incurved but the fourth was. Brushfield spots were not present but were not looked for at earlier examinations. According to Jackson *et al*,[10] the clinical diagnosis of DS is unambiguously established if 13 or more of the signs on the checklist are present. In our case, even if we include the signs that were unclear, not typical, or not examined, the clinical diagnosis of DS cannot be established according to these criteria.

During a study on the possible association of growth retardation with confined placental mosaicism, the patient showed mosaicism for trisomy 21 (73%) in the term placenta (table 2). The cytogenetic studies were extended later (table 2) and in a total of 155 blood lymphocytes and 203 skin fibroblasts only full trisomy 21 was found. No other tissues were available for study.

Three cosmid clones were selected to investigate the possibility of an internal partial deletion of 21q: two of them (c103E0669 and LLNL35A7) cover almost the entire coding region of the SIM2 gene, and cosmid

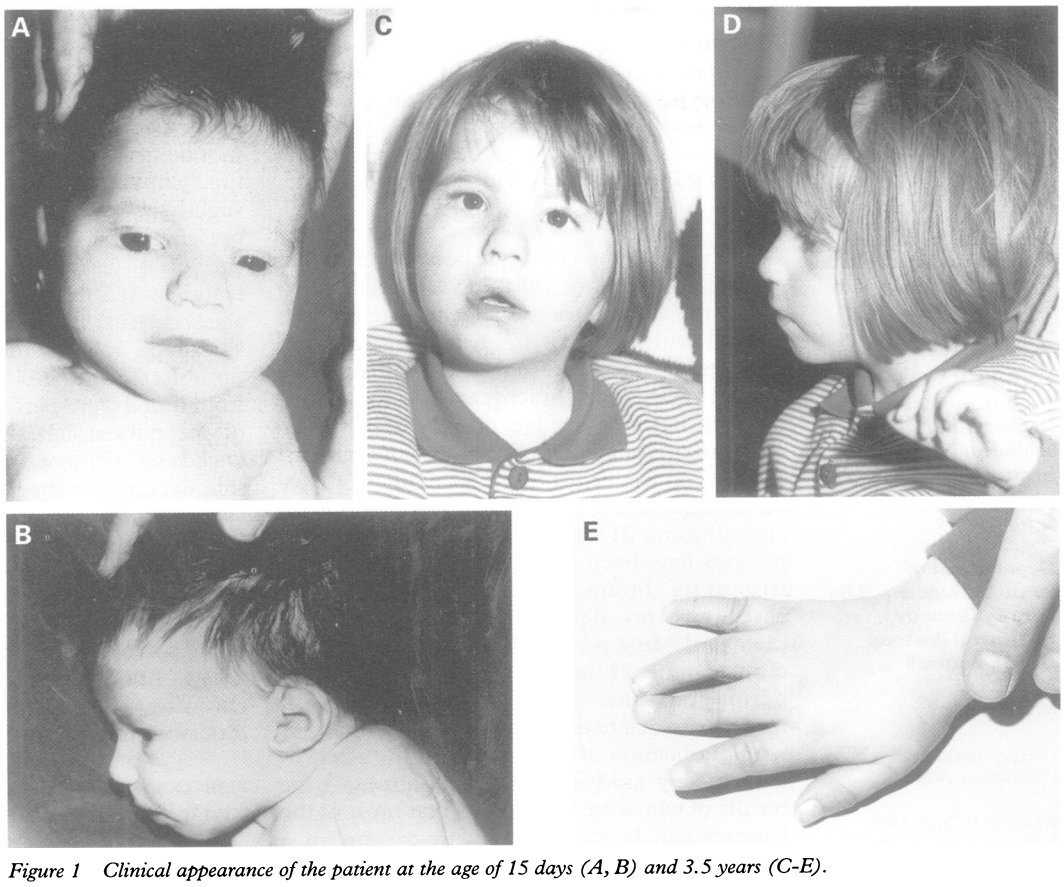

Figure 1 Clinical appearance of the patient at the age of 15 days (A, B) and 3.5 years (C-E).

Table 1 Clinical evaluation of the patient at the age of 4 years according to the Jackson checklist (the 10 most specific features are given in bold letters)

Brachycephaly	**Yes**
Oblique eye fissure	**No**
Epicanthic folds	Discrete
Blepharitis, conjunctivitis	No
Brushfield spots	No
Nystagmus	**No**
Flat nasal bridge	**Yes**
Mouth permanently open	No
Abnormal teeth	Small incisors
Protruding tongue	No
Furrowed tongue	No
High arched palate	No
Narrow palate	**No**
Folded ear	**No**
Short neck	**No**
Loose skin of neck	No
Short and broad hands	No
Short 5th finger	No
Incurved 5th finger	**4th finger**
Transverse palmar crease	No
Gap between 1st and 2nd toes	**No**
Congential heart defect	No
Murmur	No
Joint hyperflexibility	No
Muscular hypotonia	**Not informative***

* The patient had *Haemophilus influenzae* meningitis at the age of 12 months.

LLNL51G9 contains a part of the GIRK2 gene. These two genes have been localised in the DCR and are separated by more than 1 Mb. The cosmids were hybridised first on metaphase chromosomes of normal subjects to check their unique localisation in the genome, and subsequently on chromosomes from fibroblasts from the patient. All three cosmids gave a signal on the three chromosomes 21, indicating that these three probes are not deleted on any of the three chromosomes. This result was observed both on metaphase (fig 2) and interphase chromosomes.

After analysis of 20 polymorphic DNA markers along the long arm of chromosome 21, 14 markers were informative for the trisomy based on dosage analysis (table 3). On the basis of 11 informative markers, a maternal origin of the extra chromosome could be recognised (table 3). Since 16 markers along the entire length of the long arm of chromosome 21 represented "reduction to homozygosity" of maternal alleles, and as a third allele was not detected in the patient, the extra chromosome most likely originated by mitotic rather than by meiotic non-disjunction.[24]

Discussion

We report here a case of apparent trisomy 21 without the DS phenotype. We base the clinical exclusion of DS on the failure to fulfil the minimum five criteria of the Jackson sign checklist which has proven to be a very reliable tool for the diagnosis of DS. Our patient clearly presented only two of the signs on the list, while more than 13 are required for an unambiguous diagnosis. The mental retardation present in our patient could also be the result of the *Haemophilus influenzae* meningitis that occurred at the age of 12 months. These findings led us to accept that the patient, although trisomic for chromosome 21 in lymphocytes and skin fibroblasts, does not present the DS phenotype.

Table 2 Karyotype analyses

Age	*Tissue*	*46,XX/47,XX,+21*
1 day	Placenta (long term culture)	13/8, 2/33
1 week	Blood lymphocytes	0/55
2 months	Skin fibroblasts (leg)	0/100
4 years	Skin fibroblasts (arm)	0/103
4.7 years	Blood lymphocytes	0/100

In our patient the trisomy for chromosome 21 seems to have originated as a postzygotic event owing to a mitotic error, as shown by the molecular analysis. In the placental mesenchyme, which is represented by the cultured placental villi,[25] a mosaic karyotype with a cell line representing the normal karyotype of the zygote besides the trisomic cell line was detected. In contrast, in the skin fibroblasts and blood lymphocytes we did not see any normal cell line in a total of 203 and 155 cells studied, respectively. Hence, our patient can be regarded as a mosaic, the normal cell line being undetected in lymphocytes and fibroblasts. Although we did not detect mosaicism among the cells studied, because of the very mild phenotype we consider the existence of mosaicism

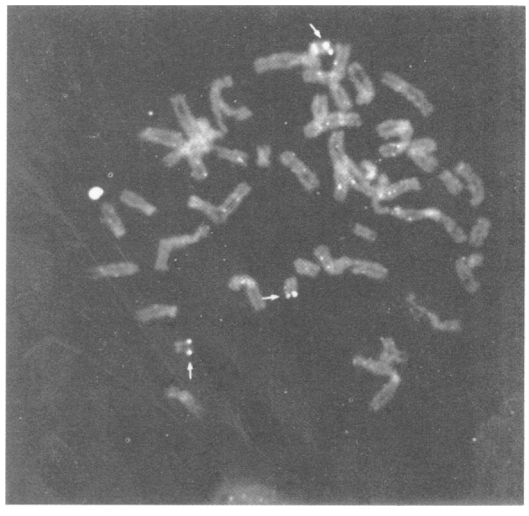

Figure 2 In situ hybridisation on metaphase chromosomes from skin fibroblasts of the patient with cosmid c103E0669. Spots of hybridisation on the three chromosomes 21 are indicated by white arrows.

Table 3 Molecular analysis

Locus cen-tel	*Genotype Fa-t21-Mo*	*Information origin/reduction*
D21S369	12-222-12	R
D21S215	11-111-12	R
D21S258	22-112-11	M
D21S120	12-111-12	R
D21S192	11-111-11	
D21S11	24-233-13	MR
D21S214	23-112-13	MR
D21S232	13-122-23	MR
D21S210	13-333-23	R
D21S226	22-112-12	MR
D21S213	23-112-12	MR
IFNAR	24-334-13	MR
D21S1283	33-113-12	MR
D21S1222	11-111-11	
D21S167	12-112-11	
D21S156	13-144-24	MR
HMG14	23-113-14	MR
D21S212	13-133-23	R
D21S171	23-233-13	R
D21S1575	23-344-14	MR

M = maternal error, R = reduction to homozygosity for the maternal alleles. The markers are shown as they appear on chromosome 21 from centromere to telomere.

in other tissues very likely, possibly with a high percentage of euploid cells.

Another possible mechanism to explain the phenotype would be a microdeletion of the DCR in the supernumerary chromosome. This situation was recently described in a 12 year old girl with moderate mental retardation, absence of classical DS dysmorphic features, trisomy 21 in lymphocytes and skin fibroblasts, but with FISH using two cosmids specific for the 21q22 band showing only two signals.[26] However, this explanation is not likely in our case since FISH analysis with three cosmid probes mapping to the DCR showed hybridisation to the three chromosomes 21. A small deletion of part of the DCR, not covered by the cosmids used, cannot be completely excluded, as this would need more than 50 hybridisations. The possible existence of genetic or other factors that could have a protective effect cannot be ruled out, but also seems unlikely since the phenomenon has not been observed before. Unfortunately, other tissues were not available for study and therefore we cannot support or rule out the possibility of mosaicism in other tissues critical for the expression of the DS phenotype.

In conclusion, our patient could be regarded as an extreme example which can be expected in a continuous spectrum of karyotype/phenotype associations in mosaic cases. Furthermore, the case underlines the need for karyotype analysis in children with mental retardation of unknown aetiology.

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